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Instability of Dinucleotide Repeats in Hodgkin's Disease

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Tumorigenesis has been shown to proceed through a series of genetic alterations involving protooncogenes and tumor suppressor genes. However, the investigation of genomic instability of microsatellites has disclosed a new mechanism for human carcinogenesis, which is involved not only in hereditary nonpolyposis colon cancer (HNPCC) but in a number of other malignancies as well. To determine whether microsatellite instability is involved in Hodgkin's disease, we screened 16 such tumors using 7 microsatellite marker loci on 6 chromosome arms 4, 5, 9p, 9q, 11, 14, and 17. Using the polymerase chain reaction method, DNA samples from the tumors and from normal peripheral blood leukocytes from each patient were compared for the allelic pattern produced at each locus. Five cases of genomic instability were identified, suggesting that this mechanism is relevant to the pathogenesis of HD. Am. J. Hematol. 57:148–152, 1998.

Key words: instability; microsatellites; Hodgkin's disease

INTRODUCTION

Hodgkin's disease (HD) is a unique pathological entity composed of a small population of malignant Reed-Sternberg (RS) cells, and a larger reactive lymphocytic population. The genetic alterations identified in molecular studies of HD represent events occurring both in RS and the reactive cell population [1]. These include immunoglobulin and T cell receptor rearrangements in some cases, and the specific integration of the EBV genome using terminal repeat probes [2,3].

The search for a molecular basis for HD has been centered on the activation of known oncogenes like the bcl-2 [4] and the ALK/Nucleophosmine fusion transcript typical of the t(2;5) found in CD-30 positive anaplastic large cell lymphoma [5,6]. Furthermore, activation of c-myc as well as p53 mutations were suggested as possible molecular explanations for HD [7–10].

In addition to the well-established role of oncogenes in the pathway to malignancy, a significant role for another class of genes involved in cancer has recently emerged. Approximately one tenth of the human genome is composed of short tandem repeats (microsatellites) made up of repetitions of two to four bases. The most common are the dinucleotide (CA/GT) repeats, about 50,000 of which are dispersed throughout the genome. Extensive instability of microsatellite repeats was recently reported in human colorectal tumors, both hereditary and sporadic, as well as in a variety of other solid tumors such as those of the pancreas, breast, liver, stomach, ovary, kidney, and the urothelium [11–13]. The genes responsible for the instability are the mutated alleles of at least four genes involved in DNA mismatch repair. These genes, termed mutators, provide a new class of genes directly involved in carcinogenesis in addition to the well-known oncogenes and tumor suppressor genes. Recent reports describe the existence of genetic instability correlating with tumorigenesis in colorectal and other carcinomas, which developed in affected members of families suffering from hereditary non-polypotic colorectal cancer (HNPCC) [13]. Linkage analysis suggested that a variant allele triggering this instability might be located on the short arm of chromosome 2 [14]. Four genes have so far been identified resulting in a similar phenotype; two are indeed located on chromosome 2 [15,16], one on chromosome 3, and one on chromosome 7 [17,18].

A significant proportion (68% of the cases) of Chronic Myeloid Leukemia cells in blast crisis demonstrate microsatellite instability, representing the first example of a

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TABLE I. Clinical Characteristics and Microsatellite Results of the 16 Patients Studied*

Patient	D11S490	D9S55	MPO	FAB	ASS	CFS	D14S431	Histology	Stage	Sex/age
1	+	+	+	+	+	+	+	MC	IVB	M/6
2	inst	−	LOH	+	+	inst	+	MC	IIIA	F/14
3	+	+	+	+	+	+	+	MC	IIIA	F/14
4	+	+	+	+	+	+	inst	NS	IIIA	M/37
5	+	+	+	+	+	+	+	MC	IIB	M/16
6	+	+	+	LOH	+	+	+	NS	IIA	M/25
7	+	−	inst	+	+	+	−	LD	IV/B	F/31
8	+	+	+	+	+	−	+	NS/MC	IIA	F/25
9	+	+	+	+	+	+	+	MC	IIB	F/18
10	+	+	+	+	+	+	+	MC	IV/B	F/8
11	+	+	inst	+	+	+	+	NS	IIA	M/15
12	+	+	+	+	+	+	+	MC	IIB	M/15
13	+	+	+	+	+	+	+	NS	IIA	F/17
14	+	+		+	+	+	0	NS	IIA	M/18
15	+	+	+	+	+	+	+			M/20
16	+	+	inst	+	+	+	+	NS	IIIB	M/20

*The different histological subtypes include mixed cellularity (MC), nodular sclerosis (NS), and lymphocytic depletion (LD). −, absence of a PCR result; +, successful amplification but no LOH or instability; inst, instability.

hematologic malignancy displaying this characteristic [19]. Microsatellite instability in 2–7 loci was found in 15 out of 16 (94%), patients with therapy-related leukemia [20]. A low frequency of instability was reported in CLL [21]. The information concerning microsatellite instability in lymphomas is scanty. Only rarely were such changes found in non-Hodgkin's lymphomas [22]. A low frequency of mutations in the mismatch repair gene, Hm1h1, was found in leukemia and lymphoma cell lines [23]. No references were found regarding HD. The question of genomic instability is of particular interest in HD since familial clustering, increased concordance in monozygous twins, and greater susceptibility in individuals with certain HLA haplotypes were reported and also because these patients are prone to the development of post-therapy malignancies [24].

In order to establish whether such mechanisms are active in Hodgkin's lymphoma, we looked into the microsatellite pattern of a group of sixteen such tumors and compared it with that of the corresponding normal DNAs. Seven microsatellite loci were examined, located on six different chromosomes.

PATIENTS AND METHODS

Tumor DNAs and corresponding normal DNAs from peripheral leukocytes were obtained according to standard procedures. To assure that the tumor pieces taken for molecular analysis contained a sufficient proportion of tumor cells, histological evaluation of a part of each piece was performed as described [25]. Thus each sample contained more than 90% tumoral material. The samples consisted of 16 HD tumors, and the clinical characteristics of the patients are presented in Table I. No familial clustering or secondary neoplasms were observed in any of these patients. PCR amplifications [26] for microsatellite analyses were performed in a total volume of 15 μl and included 200–250 ng DNA, 8–10 pmol of each primer, 0.04 μl ^{32}P-dCTP (3,000 Ci/mmol, 10.0 mCi/ml), 0.5 U Taq polymerase (Cetus, Norwalk, CT) and 166 mM of each nucleotide except for dCTP, which was only 2 mM. The reactions were submitted to 25 rounds of amplification with denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec, and elongation at 72°C for 1 min with a final elongation step at 72°C for 10 min. The microsatellite PCR products were separated by electrophoresis through a 6% sequencing gel [27,28] and exposed to X-ray films for 1 to 3 days at RT. The following seven microsatellite loci were amplified using primer sequences previously published: FABP2 (located on chromosome arm (4q28-31) [29], CFS (5q33.3-34) [30], AAS (9q34) [31], D9S55 (9p12) [32], D11S490 (11q23 3) [33], D14S43 (14q24.3) [34], and MPO (17q21-23) [35] (Table II). FABP2 represents a trinucleotide repeat (TTA), which begins at base pair 3,502 in intron B of the human intestinal fatty acid binding protein gene located on chromosome arm 4q28-q31. All the other sequences are CA repeats.

RESULTS

A total of 16 cases of HD tumors and the corresponding normal DNAs from peripheral blood leukocytes of the same patients were PCR amplified at seven microsatellite loci and their patterns compared. Table I shows the clinical characteristics of the patients and the results obtained for the seven markers.

In six patients, the allelic patterns displayed by the tumoral and the normal DNAs were not identical. Of these, four displayed instability at a single repeat, one

TABLE II. PCR Primer Sequence and Location for the Seven Markers

Marker	PCR primer sequence	Location
FABP-2	5′-AACTCAGAACAGTGCCTGAC3′	4q28-31
	5′ATTTCCCTCAAGGCTCCAGGT3	
CFS-1	5′TGTGTCCAGCCTTAGTGTGCA3′	5q33.3-34
	5′TCATCACTTCCAGAATGTGC3	
ASS	5′GGGAGCTATAAAAATGACCA3′	9q34
	5′TTAGGTCCGAAAACACAAAG3′	
D9S55	5′GAGAAAATTCCAGGCA3′	9p13
	5′GGTTGAGTCGTTCTTA3′	
D11S490	5′CACAAACATTGGCGCAT3′	11q23.3
	5′TTCTGGGTCACGGTGCTTCA3′	
D14S43	5′TGGAACACTCAGGCGA3′	14q24.3
	5′CCAGAGCCACTTTCTAC3′	
MPO	5′TCCCAGATCGCTCTACATGA3′	17q21-23
	5′CACAGCTTCAGAAGTCACAG3′	

displayed LOH at one marker, and one displayed both LOH and instability at more than one repeat.

The instability of a single repeat was in the chromosome 17q marker in three of the four cases, and in the chromosome 14 marker in the fourth case (Fig. 1). The LOH displayed by one patient was in the trinucleotide marker of chromosome 4 (Fig. 2). The patient with the multiple events displayed LOH at the chromosome 17 repeat, and instability at the chromosome 5 and 11 markers (Fig. 3).

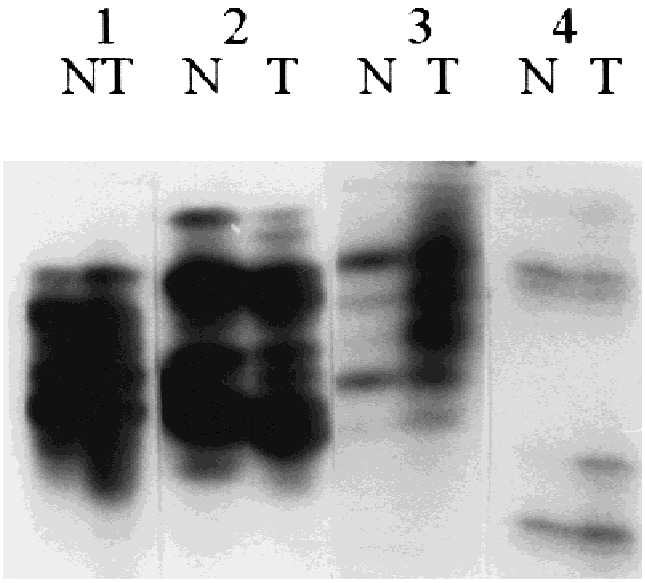

Fig. 1. Genomic instability displayed by the tumors of four patients at two loci: 1, 2, and 3 are PCR products for the MPO marker on chromosome 17, and 4 is the D14S430 marker on chromosome 14. Patient 1 corresponds to patient number 11, patient 2 to number 16, patient 3 to number 7, and patient 4 to number 4 in Table I.

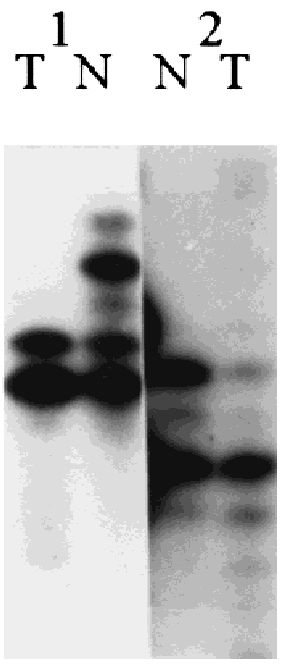

Fig. 2. LOH displayed by the tumors of two patients: Patient 1, corresponding to number 2 in Table I, at the MPO locus on chromosome 17, and patient 2, corresponding to number 6 in Table I, at the FAB locus on chromosome 4.

DISCUSSION

We describe the identification of genomic instability as manifested in alterations of the length of microsatellite sequences in five out of sixteen HD patients. Our findings provide another example in addition to those reported concerning hematological [19–23] and non-hematological malignancies [11–18]. The nature of Hodgkin's disease lesions, however, where the majority of diseased tissue is composed of reactive non-malignant cells, is of particular interest.

The observed instability may theoretically reflect either the minor population of RS cells or, alternatively, the reactive population that histologically portrays the majority of the specimen. Nevertheless, it usually represents the dominant cell population. This contrasts the findings in solid tumors where the majority of analysed cells are the malignant ones.

Hence, our findings demonstrate two important phenomena: one concerning Hodgkin's disease and the other concerning the mutator genes in general. Regarding Hodgkin's disease, the definition of the malignant vs. reactive population is very unclear. The so-called clonal population of the RS cells is not necessarily so, as was shown recently by single cell PCR analysis of VH gene sequences [36]. Others failed to show consistent bcl-2 expression in RS cells, suggesting that this oncogene expression may reflect the presence of small bystander ''reactive'' B-lymphocytes carrying the bcl-2 rearrangement, which are also found in other reactive tissues such as tonsils [4]. Moreover, the possible clonal character of

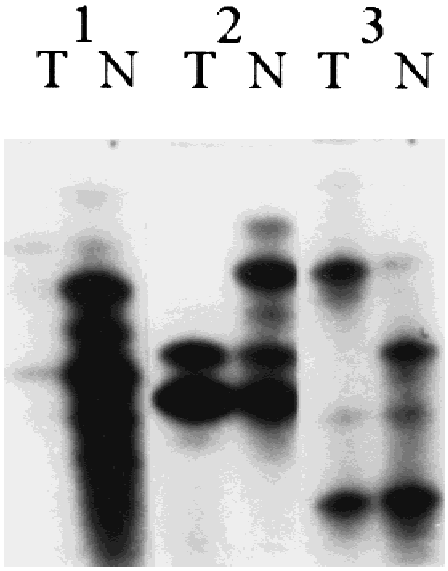

Fig. 3. Instability and LOH displayed by a single patient (number 2 in Table I) at three different loci. 1 displays genomic instability at the CFS locus on chromosome 5, 2 displays LOH at the MPO locus on chromosome 17, and 3 displays instability at the D11S490 locus on chromosome 11.

the so-called reactive population was demonstrated by Ig and TCR rearrangement in this population. Regarding the primary genetic defect in the mismatch repair mechanism, it has recently been shown by the Vogelstein group that instability of microsatellite sequences can be demonstrated in non-neoplastic cells (including peripheral blood cells) of HNPCC patients, as the mutator gene is mutated in all tissues of the body and not necessarily in the tumor tissue [37]. Therefore, the demonstration of genetic instability between 2 tissues is by itself a sign of mutator phenotype regardless of whether either tissue represents a tumor or not. These two phenomena render the exact definition of whether the microsatellite instability is related to the RS that are considered to be tumor cells or to the reactive cells, of secondary importance. We do not claim to have shown instability in the RS cells themselves, but rather the predisposition of some HD patients for genetic instability.

All the evidence presented above can, therefore, lead to the suggestion that some HD cases develop due to a mismatch repair defect. This observed instability can provide an explanation for the genetic predisposition and for the familial clustering with other hematologic malignancies observed in HD patients [24].

Furthermore, HD patients are known to have an increased risk of treatment-related neoplasms. Our findings of basic genomic instability provide an explanation for this observation. Further support for this model is provided by the findings of Abdallah et al. who described increased genetic instability in HD patients post chemotherapy as manifested in V(D)J interlocus recombination between the TCR gamma and beta genes on chromosome 7 [38]. If repair mechanisms in some HD patients are defective, the use of less mutagenic protocols should be seriously considered.

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